Introduction and Aim of the Thesis
Introduction

Chronic heart failure (CHF) is the leading cause of hospitalization in people older than age 65 [1]. In developing countries, 2-3 % of the population suffers from heart failure, but in those 70-80 years old, it occurs in 20-30 %. CHF is associated with significant morbidity and mortality attributable largely to adverse structural changes to the myocardium with related cardiac dysfunction (pump failure), arrhythmias and premature sudden death, and other associated complications such as renal disease.

Cardiac remodeling and fibrogenesis

Cardiac remodeling involves changes in size, shape, structure and physiology of the heart in response to elevated hemodynamic load and/or cardiac injury in association with neurohormonal activation [2]. Remodeling may be described physiologically or pathologically [3]. Physiological remodeling has been observed in athletes and has been termed “athlete’s heart,” whereas pathological remodeling may occur with pressure overload (e.g. aortic stenosis, hypertension), volume overload (e.g. valvular stenosis or regurgitation), or following cardiac injury (e.g. myocardial infarction, myocarditis or idiopathic dilated cardiomyopathy). In the latter, capillary network density is less and therefore not capable of supplying the greater demand of the hypertrophied myocardium. [4-6]. In each of these settings, remodeling may transition from an apparently compensatory process to a maladaptive one [7]. The transition is specifically associated with changes to both the cellular and extracellular matrix (ECM) such as myocyte hypertrophy, apoptosis or necrosis, as well as fibroblast proliferation, myofibroblast activation and development of fibrosis. All these changes are influenced by various factors including increased hemodynamic load, neurohormonal activation via endothelin and cytokines signaling, oxidative stress, matrix metalloproteinases (MMPs), and inflammatory responses involving recruitment of peripheral monocytes and macrophages (Figure 1).

Cardiac fibroblasts (CFB) are the most abundant cell type within the myocardium [8], which is critical in maintaining normal cardiac structure and ECM homeostasis [9-12]. In pathological remodeling, up-regulated CFB can result in excess matrix production and deposition of ECM proteins in the myocardium, such as collagen synthesis and deposition, which exerts adverse effects on cardiac structure and function [12-16]. In addition to being the primary source of ECM proteins, fibroblasts produce a number of cytokines, peptides and enzymes among which MMPs and tissue inhibitor of metalloproteinase (TIMPs) directly impact the ECM turnover and homeostasis [13-19]. Remodeling of the ECM plays a pivotal role in cardiac remodeling and is a key process determining the clinical course and outcome of cardiovascular diseases evolving with CHF. Recent research on the assessment and the tre-
-atment of CHF patients has expanded from an initial focus on reducing hemodynamic load to interventions that potentially modify maladaptive cellular and molecular processes.

Accumulating evidence shows that, in the pathophysiological process of CHF, molecular biomarkers could provide a unique avenue to potentially improve our capability of predicting adverse outcomes, to serve as novel drug targets, and moreover, help gauge therapeutic efficacy. Some ‘traditional’ biomarkers such as cardiac troponin, natriuretic peptides, and C-reactive protein have been studied in the patients with CHF and are currently well-established parameters in clinical practice, respectively indicating the level of cardiac myocyte damage, inflammation, ventricular remodeling, myocardial injury and renal dysfunction that occurs in CHF [20, 21]. One such emerging biomarker is Galectin-3 (Gal-3), associated mainly with myocardial fibrogenesis, and which is properly studied in the present thesis.

**The characteristics of Gal-3**

Gal-3 is a 29-35 kDa chimaera-type galectin belonging to the β-galactosidase binding lectins family, and is characterized by an extended N-terminal domain (110-130 amino acids) and a C-terminal carbohydrate recognition domain (CRD) (130 amino acids) [22, 23]. Gal-3 communicates with a variety of ECM proteins, carbohydrates (e.g., N-acetyllactosamine) as well as unglycosylated proteins such as cell surface receptors (macrophage antigens CD11b/CD18) and extracellular receptors (collagen IV) which, furthermore, modulates cell-cell adhe-
-sion signaling in the extracellular compartment [24-27].

Gal-3 is expressed in various types of cells and tissues and is important in diverse physiological and pathological processes such as immune and inflammatory responses, tumor development and progression, neural degeneration, atherosclerosis, diabetes, as well as wound repair. Gal-3 has been detected in many proliferative cells including tumor cells, eosinophils, neutrophils, activated macrophages and fibroblasts [28-31] (Figure 2). Notably, many of these cell types play active roles in the inflammatory response and the formation of fibrosis (fibrogenesis). Recent studies reveal that, as a multifunctional biomarker, Gal-3 plays a key role in the cardiac remodeling process by participating in ECM homeostasis and inflammation responses. [20]

**Gal-3 and its therapeutic implications**

Gal-3 was discovered around two decades ago [32]. It is widely distributed in several organs including the heart, lungs, liver and kidneys [33]. The role of Gal-3 in fibrosis and inflammation has been elucidated in recent years. In the healthy tissue, Gal-3 expression is absent or reduced. However, under pathological conditions, Gal-3 expression is substantially up-regulated, specifically in inflammation and fibrosis which are crucial in cardiac remodeling and renal fibrosis. In-situ hybridization and immunohistochemistry analyses show that Gal-3 is highly distributed in the fibrotic area of myocardium and co-localizes with macrophages...
An ever-growing body of experimental evidence has found that macrophage-derived Gal-3 was associated with activated myofibroblasts and subsequently increased collagen synthesis and deposition, playing an important role in regulating extracellular matrix in the damaged tissue area [30, 31, 34-41].

The first evidence showing an involvement of Gal-3 in heart failure originates from a landmark study by Sharma and colleagues [34]. The researchers demonstrated Gal-3 as a new target for intervention in the CHF. Furthermore, Henderson et al demonstrated that Gal-3 expression was markedly increased in progressive renal fibrosis. Gal-3 deficiency exhibited less renal inflammation, representing a lesser pro-fibrotic response with significant decreases in collagen production and deposition [30]. Additionally, Kalatjou et al. showed significantly increased Gal-3 expression in a kidney fibrosis model, which was amenable to a novel therapeutic strategy, Gal-3 inhibition, to attenuate fibrosis [42]. In addition to experimental studies, various clinical trials have also indicated a potential clinical utility of Gal-3 as a biomarker for prognosticating heart failure. Van Kimmenade et al firstly evaluated the prognostic and predictive value of Gal-3 as a biomarker in acute heart failure [43]. In PREVEND trial, they then demonstrated that high plasma Gal-3 levels were associated with all-cause mortality [44]. Furthermore, the DEAL-HF trial investigated the incremental value of Gal-3 over Nt-proBNP alone [45]. Finally, the HF-ACTION study revealed that Gal-3 was associated, yet not independent from NT-proBNP, with NYHA class II-IV, lower systolic blood pressure, increased creatinin, and lower maximal oxygen consumption [46].

In summary, macrophage-derived Gal-3 is associated with myofibroblast-induced collagen synthesis and deposition, playing a central role in pathophysiological cardiac remodeling and heart failure.

The aim of this thesis

As a multifunctional biomarker, Gal-3 plays an important role in detecting fibrogenesis and inflammatory processes, and is attracting widespread attention. Numerous studies have been elucidating the role of Gal-3 in the development of organ fibrosis, however, Gal-3 targeted therapy in cardiac remodeling or renal dysfunction that occurs in CHF has been given less attention.

This thesis will discuss the role of Gal-3 in fibrogenesis of cardiac remodeling and cardiac-related kidney disease. The main focus will be on Gal-3-related fibrogenesis in cardiovascular diseases. Chapter 2 and 3 of this thesis will describe the mapping of Gal-3 pathways in the pathogenesis of cardiac remodeling in CHF and illustrate its potential therapeutic target in fibrogenesis by inhibiting collagen synthesis. The main question discussed in the above two chapters is: how does Gal-3 participate in cardiac remodeling as well as further influence heart failure progression.
To further address Gal-3 targeting as a potential therapeutic candidate in cardiac remodeling, chapter 4 focuses on whether genetically or pharmacologically inhibiting Gal-3 can prevent or reverse cardiac remodeling. We will show how Gal-3 influences fibrogenesis by using different cardiac fibrosis models. Furthermore, since cardiovascular diseases are frequently accompanied with renal dysfunction, we will subsequently investigate Gal-3 targeted intervention in a hypertensive rat model (chapter 5). Moreover, we will also discuss the correlations that exist between the plasma Gal-3 levels and renal dysfunction that occurs in a well-defined clinical CHF cohort (chapter 6).
References


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